

CLAIMS

What is claimed is:

1. A method of conveying soybean sudden death resistance into non-resistant soybean germplasm comprising
5 the step of introgressing soybean sudden death resistance into non-resistant soybean germplasm using one or more nucleic acid markers for marker assisted selection among soybean lines to be used in a soybean breeding programs, said markers associated with soybean sudden death syndrome
10 resistance.

2. The method of claim 1, wherein said one or more nucleic acid marker is selected from the group consisting of
OI03₄₅₀, OI03₅₁₂, SATT309, SATT214, SATT275, SIUSAT122,
CTAAGG280, CGGAGA300, ATGCGA190, AGGCAC310, CCACCA120,
15 CCCTC220, ACGCAT80, OG13₄₉₀, Bng122, SATT163, SATT38, A112I,
OE04₄₅₀, OE02₁₀₀₀, SATT130, OC01₅₀₀, OO04₁₀₇₅, SATT9, OO05₂₅₀,
K455D, OP13₅₀₀, OG01₁₀₀₀, SZ19₅₀₀, SATT71, OW15₁₀₀₀, AO85, OA12₁₀₀₀,
BLT65, CCAAGC309, CCCATG349, CCGAAC400, CCGAAC401,
CCCATG350, CCAAGC310, OW15500, OD04₅₀₀, A063I and SAT40.

20 3. The method of claim 1, wherein said marker assisted selection comprises the use of an analysis technique selected from the group consisting of RAPD analysis, RFLP analysis, microsatellite analysis, and AFLP analysis.

4. The method of claim 1, wherein the source of the sudden death syndrome resistance comprises a soybean strain selected from the group consisting of Forrest or a descendant thereof, Essex or a descendant thereof, Pyramid or a descendant thereof, Ripley or a descendant thereof, Jack or a descendant thereof, PI520733 or a descendant thereof, PI567507B or a descendant thereof, PI567.365 or a descendant thereof, PI567.446B or a descendant thereof, PI567.373B or a descendant thereof, PI90763 or a descendant thereof, PI209332 or a descendant thereof.

5. A method of reliably and predictably introgressing soybean sudden death syndrome resistance into non-resistant soybean germplasm comprising using one or more nucleic acid markers for marker assisted selection among soybean lines to be used in a soybean breeding program, wherein the nucleic acid markers are: OI03₄₅₀, OI03₅₁₂, SATT309, SATT214, SATT275, SIUSAT122, CTAAGG280, CGGAGA300, ATGCGA190, AGGCAC310, CCACCA120, CCCTC220, ACGCAT80, OG13₄₉₀, Bng122, SATT163, SATT38, A112I, OE04₄₅₀, OE02₁₀₀₀, SATT130, OC01₅₀₀, OO04₁₀₇₅, SATT9, OO05₂₅₀, K455D, OP13₅₀₀, OG01₁₀₀₀, SZ19₅₀₀, SATT71, OW15₁₀₀₀, AO85, OA12₁₀₀₀, BLT65, CCAAGC309, CCCATG349, CCGAAC400, CCGAAC401, CCCATG350, CCAAGC310, OW15500, OD04₅₀₀, A063I and

SAT40, and introgressing said resistance into said non-resistant soybean germplasm.

6. The method of claim 5, wherein said marker assisted selection comprises the use of an analysis technique selected from the group consisting of RAPD analysis, RFLP analysis, microsatellite analysis, and AFLP analysis.

7. The method of claim 5, wherein the source of the sudden death syndrome resistance comprises a soybean strain selected from the group consisting of Forrest or a descendant thereof, Essex or a descendant thereof, Pyramid or a descendant thereof, Ripley or a descendant thereof, Jack or a descendant thereof, PI520733 or a descendant thereof, PI567507B or a descendant thereof, PI567.365 or a descendant thereof, PI567.446B or a descendant thereof, PI567.373B or a descendant thereof, PI90763 or a descendant thereof, and PI209332 or a descendant thereof.

8. A quantitative trait locus associated with resistance to soybean sudden death syndrome, said locus mapping to a linkage group in the soybean genome, said linkage group selected from the group consisting of G, N, C2, B, D, A2, and C1.

9. A quantitative trait locus of claim 8, said locus mapping to linkage group G and mapped by one or more of the

markers OI03₄₅₀, OI03₅₁₂, SATT309, SATT214, SATT275, SIUSAT122, CTAAGG280, CGGAGA300, ATGCGA190, AGGCAC310, CCACCA120, CCCTC220, ACGCAT80, OG13₄₉₀, Bng122, SATT163, SATT38, A112I, OE04₄₅₀, OE02₁₀₀₀, and SATT130.

5 10. A quantitative trait locus of claim 8, said locus mapping to linkage group N and mapped by one or more of the markers OC01₅₀₀, OO04₁₀₇₅ and SATT9.

10 11. A quantitative trait locus of claim 8, said locus mapping to linkage group C2 and mapped by one or more of the markers OO05₂₅₀, K455D and OP13₅₀₀.

 12. A quantitative trait locus of claim 8, said locus mapped by one or more of the markers OG01₁₀₀₀, SZ19₅₀₀ and SATT71.

15 13. A quantitative trait locus of claim 8, said locus mapping to linkage group A2 and mapped by one or more of the markers OW15₁₀₀₀, AO85, OA12₁₀₀₀, BLT65, CCAAGC309, CCCATG349, CCGAAC400, CCGAAC401, CCCATG350, CCAAGC310, OW15500, and OD04₅₀₀.

20 14. A quantitative trait locus of claim 8, said locus mapping to linkage group C1 and mapped by one or more of the markers A063I and SAT42.

 15. An isolated and purified genetic marker associated with soybean sudden death (SDS) resistance in soybeans, said

marker mapping to a linkage group in the soybean genome, said linkage group selected from the group consisting of G, N, D, C2, B, A2, and C1.

16. The genetic marker of claim 15, further defined as
5 an isolated and purified polynucleotide comprising a base sequence that is identical or complementary to a segment of at least 10 contiguous bases of SEQ ID NOS: 1-20, wherein said polynucleotide hybridizes to the sequence of SEQ ID NOS: 1-20.

10 17. The genetic marker of claim 16, further defined as comprising a base sequence that is identical or complementary to a segment of at least 25 contiguous bases of SEQ ID NOS: 1-20.

15 18. The genetic marker of claim 17, further defined as comprising a base sequence that is identical or complementary to a segment of at least 100 contiguous bases of SEQ ID NOS: 1-20.

19. The genetic marker of claim 18, further defined as having a base sequence that is identical or complementary to
20 SEQ ID NOS: 1-20.

20. An isolated and purified polynucleotide consisting of 10 to 1500 nucleotides which hybridizes preferentially to RNA or DNA of soybean and not to non-soybean organisms,

wherein said polynucleotide sequence is or is complementary to a nucleotide sequence consisting of at least ten consecutive nucleotides from a nucleotide sequence selected from the group consisting of SEQ ID NOS: 1-20.

5 21. A probe comprising a polynucleotide of claim 20 and a detectable moiety.

22. An improved soybean plant, seed or tissue culture produced by the method of claim 1.

23. An improved soybean plant, seed or tissue culture
10 produced by the method of claim 2.

24. An improved soybean plant, and parts thereof, which evidences a resistance response to soybean sudden death syndrome (SDS) comprising a genome, homozygous with respect to genetic alleles which are native to a first parent and
15 nonnative to a second parent of the improved soybean plant, wherein said second parent evidences significantly less resistant response to SDS than said first parent and said improved plant comprises alleles from said first parent that evidences resistance to SDS in hybrid combination in at least
20 one locus selected from: a locus mapping to linkage group G and mapped by one or more of the markers OI03₄₅₀, OG13₄₉₀, Bng122, SATT163, SATT38, A112I, OE04₄₅₀, OE02₁₀₀₀ and SATT130; a locus mapping to linkage group N and mapped by one or more

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of the markers OC01₅₀₀, OO04₁₀₇₅ and SATT9; a locus mapping to linkage group C2 and mapped by one or more of the markers OO05₂₅₀, K455D and OP13₅₀₀; a locus mapped by one or more of the markers OG01₁₀₀₀, SZ19₅₀₀ and SATT71; a locus mapping to linkage group A2 and mapped by one or more of the markers OW15₁₀₀₀, A085 and OA12₁₀₀₀; or a locus mapping to linkage group C1 and mapped by one or more of the markers A063I and SAT42, said resistance not significantly less than that of the first parent in the same hybrid combination, and yield characteristics which are not significantly different than those of the second parent in the same hybrid combination.

25. The inbred soybean plant of claim 24 comprising each of: a locus mapping to linkage group G and mapped by one or more of the markers OI03₄₅₀, OG13₄₉₀, Bng122, SATT163, SATT38, A112I, OE04₄₅₀, OE02₁₀₀₀ and SATT130; a locus mapping to linkage group N and mapped by one or more of the markers OC01₅₀₀, OO04₁₀₇₅ and SATT9; a locus mapping to linkage group C2 and mapped by one or more of the markers OO05₂₅₀, K455D and OP13₅₀₀; a locus mapping to linkage group D and mapped by one or more of the markers OG01₁₀₀₀, SZ19₅₀₀ and SATT71; a locus mapping to linkage group A2 and mapped by one or more of the markers OW15₁₀₀₀, A085 and OA12₁₀₀₀; or a locus mapping to linkage group C1 and mapped by one or more of the markers

A063I and SAT42, and having improved resistance to SDS when compared to a substantially identical inbred soybean plant not comprising said loci.

26. A soybean plant, and parts thereof, as claimed in claim 24 comprising the progeny of a cross between first and second inbred lines, alleles conferring resistance to sudden death syndrome (SDS) being present in the homozygous state in the genome of one or the other or both of said first and second inbred lines such that the genome of said first and second inbreds together donate to the hybrid a complement of alleles necessary to confer the resistance to SDS.

27. A sudden death syndrome resistant hybrid and parts thereof formed with the improved soybean plant according to claim 25.

28. A soybean plant and parts thereof formed by selfing the sudden death syndrome resistant hybrid of claim 27.

29. A method for the production of an inbred soybean plant adapted for conferring, in hybrid combination with a suitable second inbred, resistance to sudden death syndrome (SDS) comprising:

- (a) selecting a first donor parental line possessing the desired SDS resistance having at least one of the resistant loci selected from: a locus mapping

to linkage group G and mapped by one or more of the markers OI03₄₅₀, OG13₄₉₀, Bng122, SATT163, SATT38, A112I, OE04₄₅₀, OE02₁₀₀₀ and SATT130; a locus mapping to linkage group N and mapped by one or more of the markers OC01₅₀₀, OO04₁₀₇₅ and SATT9; a locus mapping to linkage group C2 and mapped by one or more of the markers OO05₂₅₀, K455D and OP13₅₀₀; a locus mapping to linkage group D and mapped by one or more of the markers OG01₁₀₀₀, SZ19₅₀₀ and SATT71; a locus mapping to linkage group A2 and mapped by one or more of the markers OW15₁₀₀₀, A085 and OA12₁₀₀₀; or a locus mapping to linkage group C1 and mapped by one or more of the markers A063I and SAT42, and crossing same with a second parental line which is high yielding in hybrid combination, to produce a segregating plant population;

- (b) screening the plant population for identified chromosomal loci of one or more genes associated with the resistance to the SDS trait; and
- (c) selecting plants from said population having said identified chromosomal loci for further screening until a line is obtained which is homozygous for

resistance to SDS at sufficient loci to give
resistance to SDS in hybrid combination.

30. A method for identifying soybean sudden death
syndrome (SDS) resistance or soybean cyst nematode resistance
5 in a soybean plant using a SDS resistance gene, a SCN
resistance gene or DNA segments having homology to a SDS
resistance gene or to a SCN resistance gene, the method
comprising the steps of:

(a) probing nucleic acids obtained from the soybean
10 plant with a probe derived from said SDS resistance
gene or from said SCN resistance gene or from said
DNA segment having homology to said SDS resistance
gene or to said SCN resistance gene; and

(b) observing hybridization of said probe to said
15 nucleic acids, the presence of said hybridization
indicating SDS or SCN resistance in said soybean
plant.

31. The method of claim 30, further comprising using
the probe derived from a known resistance gene to probe plant
20 nucleic acids to look for mapping of the probe to loci for
SDS or SCN resistance, the presence of said mapping
indicating the presence of SDS or SCN resistance.

32. A method of reliably and predictably introgressing soybean cyst nematode resistance into non-resistant soybean germplasm comprising using one or more nucleic acid markers for marker assisted selection among soybean lines to be used in a soybean breeding program, wherein the nucleic acid markers are OI03₄₅₀, OG13₄₉₀, Bng122, SATT163, SATT37, OG01₁₀₀₀ SZ19₅₀₀, SATT71, OW15₁₀₀₀, A085 and OA12₁₀₀₀, and introgressing said resistance into said non-resistant soybean germplasm.

33. A quantitative trait locus associated with resistance to soybean cyst nematode, said locus mapping to linkage group B and mapped by one or more of the markers OG01₁₀₀₀ SZ19₅₀₀ and SATT71.

34. An improved soybean plant, seed or tissue culture produced by the method of claim 32.

35. An improved soybean plant having soybean cyst nematode (SCN) resistance, the genome of which contains genes associated with said SCN resistance at a locus mapping to linkage group B and mapped by one or more of the markers OG01₁₀₀₀ SZ19₅₀₀ and SATT71.

36. An isolated and purified soybean rfs1 gene, said gene capable of conveying *Fusarium solani*-infection resistance to a non-resistant soybean germplasm, said gene located within a quantitative trait locus mapping to linkage

group G and mapped by genetic markers OI03₅₁₂ and Bng122, said gene located along said quantitative trait locus between said markers.

37. An isolated and purified soybean rft1 gene, said gene capable of conveying *Fusarium solani* toxin resistance to a non-resistant soybean germplasm, said gene located within a quantitative trait locus mapping to linkage group G and mapped by genetic markers OI03₅₁₂ and SATT309, said gene located along said quantitative trait locus between said markers.

38. A method of determining soybean sudden death syndrome resistance in a soybean plant in a greenhouse setting, the method comprising the steps of:

- (a) inoculating soil with a low density inoculum of *Fusarium solani*;
- (b) planting a soybean plant in said inoculated soil;
- (c) growing said plant in said soil for a predetermined period of time in a greenhouse;
- (d) isolating *Fusarium solani*-infected tissue from said plant;
- (e) culturing said infected tissue for a period of time sufficient to allow for fungal colony forming unit growth;

(f) statistically determining disease severity and/or infection severity in said plant using the number of said fungal colony forming units; and

(g) comparing said disease severity and/or said infection severity to disease severity and/or infection severity data from genetic markers associated with soybean sudden death syndrome resistance to identify a correlation, the presence of a statistically significant correlation indicating the presence of soybean sudden death syndrome resistance in said soybean plant.

39. The method of claim 38, wherein said low density inoculum of *Fusarium solani* comprises an inoculum density of about 3×10^3 spore/cm³ soil.

40. The method of claim 38, wherein said *Fusarium solani*-infected plant tissue comprises root tissue.

41. A method of characterizing resistance to soybean sudden death syndrome in a soybean plant, the method comprising the steps of:

(a) isolating roots from a soybean plant infected by *Fusarium solani*;

- (b) culturing the root on a culture plate including a restrictive growth medium that provides for slow fungal growth and restricted bacterial growth;
- (c) determining root infection severity by statistically evaluating the number of *Fusarium solani* colony forming units on said culture plate; and
- (d) characterizing resistance to soybean sudden death syndrome in said soybean plant based on said determined root infection severity.

42. The method of claim 41, wherein said characterization of resistance to soybean sudden death syndrome further comprises determining a level of resistance to soybean sudden death in the soybean plant.